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TALPID3 in Joubert Syndrome and related ciliopathy disorders

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Abstract

TALPID3 (KIAA0586) is a centrosomal, protein which has specific functions during centriole maturation during the formation of the centrosomal dependent organelle, the cilia, as well as less well understood roles in the cytoskeleton and during cell polarisation. Cilia are an essential component of signal transduction during embryonic development and the loss of TALPID3 function in humans can cause both severe lethal and mild cilia-related developmental disorders known as 'ciliopathies' the most common being Joubert syndrome. TALPID3 related ciliopathies affect the development of multiple organ systems including the brain, skeleton, eyes, lungs and liver. The consequences of TALPID3 dysfunction outside of the cilia and the implications for human diseases, is less well understood.

Introduction

When the *talpid*^{3} chicken mutant was first discovered by Donald Ede at Wye Agricultural College, UK in the 1960s, during a preliminary investigation into chick hatchability [1] it was never anticipated that this peculiar mutant would one day contribute to understanding rare

human diseases. Fortuitously Dr Ede, a previous student of Prof. C.H. Waddington, was an excellent anatomist and developmental biologist, with a particular interest in embryonic lethal developmental mutants with a disruption of pattern [2]. Donald immediately understood the significance of the severely disrupted anatomy of the *talpid*^{3} embryos, which was particularly evident in the polydactylous limbs and head [3,4]. His work and the discovery of *talpid*^{3}, came at a timely moment in developmental biology, just as the chicken limb was becoming an important paradigm for understanding the induction of pattern in the developing embryo, in fact preceding the discovery of the cellular 'organiser' of digit number and identity [4]. *Talpid*^{3} was to play an important role in understanding the developmental mechanisms which pattern the developing limb and in turn, forty years later, research on polydactylous mice helped established that a loss of cilia underpinned the unusual *talpid*^{3} phenotype [4].

Talpid^{3} chicken embryos are characterised by gross malformations of the brain, face, skeleton and limbs and have been a focus of many studies over several decades, in an attempt to understand the basis of their aberrant anatomy [3,4]. The *TALPID3* gene (also known as *KIAA0586*), has now also been deleted in different animal and cell models, generating a well defined catalogue defects that occur upon TALPID3 loss of function (Table 1, Figure 1A, B). Primarily, a loss of *TALPID3* causes a failure of cilia formation [5,6] and thus *TALPID3* mutants fall into a class of developmental phenotypes known as 'ciliopathies'. Cilia are centrosome dependent organelles which project from the cell surface (Figure 1A). Cilia can be both actively motile such as ependymal cell cilia which line the ventricles of the brain, or 'primary' non-motile cilia, which are found on most cell types and are essential for some signalling pathways, in particular Hedgehog signalling (Figure 1A)[7]. Most recently, this in-depth understanding of the *TALPID3* phenotype has aided identification of human ciliopathy patients with mutations in *TALPID3/KIAA0586* [8-16]. Analyses of TALPID3 at the molecular level, meanwhile, have revealed that this centrosomal protein [17] has a role within mother

centriole maturation prior to initiation of ciliogenesis [18] although there is evidence that TALPID3 may also have other non-ciliary centrosomal roles such as within organisation of the polarised cytoskeleton. [5,6,15] as well as possible roles independent of the centrosome (Figure.1A).

TALPID3- sequence, homology, domains and interactions

Vertebrates have a single *TALPID3* gene, encoding a centrosomal protein of between 1520-1644aa (Figure 2), which localises specifically to the distal ends of both the mother and daughter centrioles which comprise the centrosome complex (Figure3A, 3C, C', [5,17-21]). It is one of the least abundant centrosomal proteins [22] and has an asymmetrical distribution, being more highly localised on the mother centriole (Figure 3A,[21]). TALPID3 does not show homology to other protein families, and its structure remains elusive. While the *TALPID3* sequence is not highly conserved (the human TALPID3 protein is approximately 70% identical to mouse and 45% to chicken), TALPID3 proteins do contain conserved coiled coil domains approximately 450-650aa from the N' terminal (Figure 2, pink boxes), one of which is essential for function and which mediates both localisation of TALPID3 to the centrosome and the majority of known TALPID3 protein-protein interactions [blue asterisk; 4,18,19, 21, 23-27]. Deletion of this domain causes a *TALPID3* null phenotype in chicken, mice, zebrafish and human cells [5,18,19, 24, 27]. There is evidence that the TALPID3 coiled coil domains specifically mediate the maturation of the mother centriole through loss of daughter centriolar proteins and assembly of the basal body distal appendages prior to ciliogenesis [27]. A less well defined portion of C'terminal end of TALPID3 is also essential for function [5,27]. This region binds to the PKA regulatory subunit PKARIIβ, through which is it proposed to mediate Hedgehog signalling via PKA phosphorylation of the Gli proteins [28]. In addition the

C'terminal may also mediate ciliogenesis through CP110 removal and ciliary vesicle docking [27].

A Ciliopathy- the *TALPID3* Phenotype in Models and Man

Other than a few species-dependent exceptions, the phenotypes of the *TALPID3* null animal models are very similar. All are recessive and embryos homozygous for the *TALPID3* mutations lack cilia (including motile cilia) and are embryonic lethal (Table 1, Supplementary Table 1, Figure 1A);[1,5,19,24,28]. The developmental abnormalities exhibited by *TALPID3* null model embryos of all species, such as hypotelorism, holoprosencephaly, unpatterned polysyndactyly limbs and others (Table 1) are highly characteristic of vertebrate ciliopathy mutants which lack primary cilia and have disrupted Hedgehog signalling [7]. Since 2015 a number of human patients with mutations in *TALPID3/KIAA0586* have been described providing an allelic series of *TALPID3/KIAA058* mutations (Figure 2); [8-16]. In human patients rare homozygous mutations at c.230C>G, p.Ser77* (null) and c.1815G>A in *KIAA0586* (the human ortholog of *TALPID3*), which appear to disrupt the TALPID3 protein before or around the essential coiled coil domain, cause a reduction of cilia on patient cells (c.1815G>A) and gestational and perinatal lethal phenotypes, respectively. Like the animal models, these null human alleles are associated with polydactyly and micromelia [9]. Additional phenotypes in these patients included cleft palate and hydrocephalus (c.230C>G, p.Ser77*), short ribs, abnormal tongue development and brain malformations (c.1815G>A), suggestive of Short-Rib Polydactyly ciliopathy syndrome. The similarity with the null *TALPID3* animal models was unequivocal, demonstrating a conservation of *TALPID3/KIAA0586* function in humans.

The majority of human patients with mutations in *TALPID3/KIAA0586*, are however, viable and although they demonstrate challenging phenotypes associated with ciliopathy disorders these are at the mildest end of the ciliopathy spectrum [8, 10-15]. Joubert syndrome, the most common and mild syndrome in the ciliopathy spectrum caused by mutations in *KIAA0586*, is a genetically heterogenous disorder which can be caused by mutations in at least 34 different loci, including *TALPID3/KIAA0586* (JBTS23; [29]. Diagnosis of Joubert syndrome is based on cerebellum and hindbrain malformations, specifically the 'molar tooth sign' observed on an axial MRI [Figure 1B. 29,30]. As the cerebellum is particularly susceptible to disruption in Hedgehog signalling [31], it is hypothesised that the molar tooth sign is due to a loss of Hedgehog dependent development of the cerebellar vermis resulting in fewer efferent fibres exiting the cerebellum via the paired superior cerebellar peduncles. Additionally it has also been proposed that there is a loss of decussation (crossing of the midline) of superior cerebellar peduncle neuronal tracts [29,30]. As the cerebellum normally coordinates voluntary movements such as posture, balance, coordination and speech as well as eye movement and breathing [31], common clinical characteristics associated with Joubert syndrome include hypotonia, abnormal eye movements, abnormal breathing patterns, slow speech, development and intellectual impairment [29] all of which have been described in patients with mutations in *KIAA0586* [8,10-16]. In order to model Joubert syndrome through loss of *Talpid3*, a mouse with a conditional deletion of *Talpid3* in the central nervous system, has recently been generated [32]. This had a surprisingly specific effect on the development of the cerebellum and little effect, other than hydrocephaly in the rest of the central nervous system. The phenotype of these mice closely recapitulated many aspects of Joubert syndrome [32]. As well as severe hypoplasia and a lack of foliation of the cerebellar hemispheres, there was loss of decussation of the superior cerebellar peduncles. In addition, both the Purkinje cell layer and the granule cell layer were affected by a loss of *Talpid3*; proliferation and normal organisation

of granule layer cells were compromised, while the Purkinje cell layer demonstrated abnormal dendritic arborisation and a widespread disruption of the cerebellar circuitry [32]. This mouse model demonstrated significant ataxia, supporting its relevance as a model for Joubert syndrome, but happily the mice were able to both feed and groom.

The *TALPID3* Gene: a Genotype to Phenotype Conundrum

Joubert syndrome is considered a pleiotropic condition and patients commonly exhibit other ciliopathy phenotypes including retinal dystrophy, kidney disease, coloboma, liver fibrosis, encephalocele and polydactyly [33]. Despite *TALPID3/KIAA0586* null phenotype clearly acting pleiotropically in both human and animal models, Joubert syndrome caused by mutations in *TALPID3/KIAA0586* is only rarely associated with other ciliopathy related phenotypes (Figure 1B). These include a limited number of examples of coloboma [10,16] atrial-septal defects [10,15] polydactyly, sensorineural hearing loss [10], skeletal dysplasia of the thorax [12] and mild respiratory and liver dysfunction [12]. The difference in severity of phenotypes both between the severe and mild ciliopathy phenotypes caused by *TALPID3/KIAA0586* mutations as well as between different Joubert loci, may be due both to the heterogenous nature of the human mutations and/or to the tissue-specific requirements for *TALPID3/KIAA0586*, cilia or Hedgehog signalling. It is clear that mutations in the 5' end of *TALPID3/KIAA0586* are likely to cause a ciliopathy phenotype (Figure 2) and that mutations that disrupt the essential coiled-coil domain are more likely to cause a severe ciliopathy phenotypes, with the exception of the c.428delG p.Arg143Lysfs*4 mutations.

The c.428delG p.Arg143Lysfs*4 mutation is a particularly prevalent mutation accounting for 34/49 known compound heterozygous mutations, and while it would be predicted to disrupt the coiled coil domains causing a loss of TALPID3/KIAA0586 function, there are two reported

cases of Joubert syndrome patients and one reported healthy individual, homozygous for the mutation [10,13,14]. This suggests the c.428delG p.Arg143Lysfs*4 mutation is instead likely to function as a hypomorphic allele, possibly through the use of an alternative start codon downstream of the c.428delG p.Arg143Lysfs*4 mutation which may produce a functional shortened protein (Figure 2, purple arrow; NM_001244193/NP_001231122); [13]. The disparity between the severe embryonic lethal phenotype observed in null *TALPID3* embryos and the relativity mild Joubert phenotype observed in the majority of *TALPID3/KIAA0586* compound heterozygous mutations including those patients with a c.428delG p.Arg143Lysfs*4, support the hypothesis that this is a hypomorphic allele. TALPID3 is a low abundance protein [22] and the disparity between null and hypomorphic alleles perhaps suggests that very little active TALPID3 protein is required to fulfil its function in the cell.

More than a Ciliopathy- TALPID3 in the Centrosome and Cytoskeleton

The *TALPID3* null phenotype is driven not only by a loss of cilia but by multiple mechanistic failures within the centrosome and possibly cytoskeleton, prior to ciliogenesis [5, 6, 15, 18, 26, 27]. The centrosome is a highly complex, structured and dynamic organelle, comprising of two centrioles surrounded by centriolar material and the centriolar satellites [17,20]. The centrioles, known as the mother and daughter centrioles are structurally different and have different and asymmetric protein compositions [20]. Centrioles lacking *TALPID3* exhibit a number of abnormalities; *TALPID3* null centrioles fail to migrate normally within the cell, are overly elongated, have a loss of orientation and are unable to dock with the ciliary vescile. Critically, in *TALPID3* null cells, mother centrioles fails to undergo maturation prior to ciliary vesicle docking and ciliogenesis. Detailed analysis of this process has shown that centrioles lacking TALPID3 do not undertake removal of daughter centriolar proteins and subsequently

also fail to acquire mother centriolar proteins during maturation, including normal Distal Appendage (DA) proteins and structure [Figure 3B; 5,6,18,27], although some disorganised DA proteins have been shown to be present in human and chicken TALPID3 null cells [9, 15]. In addition around the centrioles there is an increased number and disorganisation of the centriolar satellites. Increasingly the search to understand the biological function of TALPID3 has turned to proteomics combined with super resolution microscopy and genome editing technology to assess the hierarchical and dynamic nature of TALPID3 protein interactions within the centrosome [27,34]. TALPID3 localises to the distal ends of both the mother and daughter centrioles and directly interacts with numerous centrosomal proteins including PCM1, MIB1, CP110, CEP290, CEP120, C2CD3, MACF1, CEP97/KIF24, RAB8a, PKARIIβ [18,21,25,26,27,28,34]. PCM1 is key controller of the abundance of TALPID3 on the mother centrioles by sequestering the E3 Ligase MIB1 to the centriolar satellites, which would otherwise promotes the poly-ubiquitylation of TALPID3. TALPID3 is recruited to the distal centrioles through reciprocal interactions with C2CD3 and CEP120; the loss of the any one of these two partners leads to a failure of ciliogenesis and Joubert syndrome [15, 34]. Once at the distal end of the mother centriole, TALPID3 and C2CD3 play a central role in the maturation of the mother centriole, controlling first the removal of daughter centriolar proteins and then the recruitment of proteins during the assembly of the DA [21,34] (Figure 3A). While the Sub Distal Appendage (SDA) appears to remain intact, TALPID3 is also known to interact with MACF1, a SDA protein and other proteins between the DA and SDA are mislocalised (CEP19). TALPID3 also interacts with CP110 which must be removed from the distal centriole to allow prior to the recruitment of ciliary vesicles, which is dependent on RAB8a (Figure 3A) [25, 27]. Recent papers [27,34] have utilised human *TALPID3/KIAA0586* mutations to inform proteomic approaches to establish potential residues and uncharacterised domains that the TALPID3 protein used to control the dynamic and transient actions of the distal centriolar

interacting protein network. The distinctive multiple roles of TALPID3 within the distal centriole are not generic to all proteins of the distal centriolar protein network- nor even TALPID3's binding partners such as C2CD3. *C2CD3* is a particularly interesting example; a null mutation in *C2CD3* also causes an embryonic lethal chicken mutant, *talpid*^{2} [3]. While *talpid*^{3} and *talpid*^{2} have overlapping phenotypic features (polydactyly) and both cause a loss of OFD1 at the distal end of the centriole [27], they also have distinctive differences; hyperteloism in *talpid*^{2}, hypoteloism in *talpid*^{3} [3]; a loss of TALPID3 causes elongation of the centrioles, while a loss of C2CD3 causes a shortening; TALPID3 null cells have an abnormal actin cytokeleton, while null C2CD3 cells do not [5, 34]. Thus while TALPID3 and C2CD3 have overlapping and co-ordinated functions they also have distinctive functions from each other, which lead to diverse phenotypes in null situations.

Actin cytoskeleton defects are observed in both chickens and human *TALPID3* null cells and *TALPID3* null animal models also demonstrate cell polarity defects (Table1) [5, 27] including a recent demonstration of the disruption of cell polarity in photoreceptor cells [35]. The centrosome may also be nucleation centre for the actin cytoskeleton [40] but it is currently unknown whether the actin cytoskeleton is directly mediated by TALPID3, or perhaps through interactions with other proteins such a MACF1 (Microtubule Actin Crosslinking Factor 1; [25]). This is, however, an important distinction between the *TALPID3* null phenotype and other ciliopathy mutants which have normal centrosomes but lack cilia for other reasons. While superficially ciliopathy phenotypes are overwhelming due to the loss of normal Hedgehog signalling, fundamentally the phenotype of *TALPID3* null mutants is also underpinned by a different and abnormal cell biology, with different, although subtle consequences for the embryo and perhaps for patients.

Conclusion

The *talpid*^{3} legacy is far from complete. We have, as yet, only a partial understanding of the action of TALPID3 within the centrosome with hints that there are dynamic and multiple roles for TALPID3. We still have little understanding of the action of TALPID3 in regulation of the cytoskeleton or cell polarity. It is likely that these outstanding questions are in fact one and the same and that in combining the power of human genetics with proteomics, microscopy and model systems we will generate new insights into this enduring biological problem and illuminate the connection between cell biology and phenotype, towards, we hope, informing the development of future therapies for those living with Joubert syndrome and other ciliopathies.

In memory of Dr Donald A. Ede, discoverer of the talpid^{3} *chicken mutant 4*th *May 1926-22*nd *August 2018.*

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*Joubert patients with TALPID3 mutations do not have retinal degeneration but this paper highlights that this is a progressive disease in in animal models thus it may be important to monitor human patients in the future.

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PHENOTYPE	Ciliopathy Phenotype	TALPID3 Animal Model	Short-Rib Polydacyly	Joubert Syndrome	Hedgehog related	Cell polarity related
CELLULAR DEFECTS	yes	1, 2, 3, 5, 6, 7	yes			yes
Cilia absent/reduced, smaller		1, 2, 3, 5, 6, 7	yes	Yes [11]		yes
Abnormal centrosome location		1, 2, 3, 5, 6, 7				yes
Increase centriolar satellites		1,7				
Increased centriole length		1,7				
Failure of Distal Appendage		1 (reduced), 7	CEP164 seen			
Abnormal actin cytoskeleton		1, 7				yes
Abnormal Hedgehog signalling	yes	1,2,3,5	yes		yes	
FACE AND HEAD DEFECTS	yes	5	yes	yes	yes	yes
Frontonasal prominence	yes	1,2	yes	yes	yes	
Hypotelorism	yes	1,2,5		yes	yes	
Midface shortening	yes			yes		
Long philtrum				yes		
Mandibular prominence	yes	1,2		yes	yes	
Micrognathia	yes	1		yes	yes	
Retrognathia	yes	1	yes			
Cleft tongue	yes		yes			
Abnormal dentition	yes		yes	yes	yes	
Maxillary prominence		1,2	yes		yes	
Cleft palate	yes		yes			
Abnormal dentition	yes			yes	yes	
CNS DEFECTS	yes	1,2,3,5		yes	yes	
Hydrocephaly	yes	3	yes	yes		
Enlarged ventricles	yes	3	yes	yes		
Forebrain	yes	2,3	yes		yes	
Holoprosencephaly	yes	1,2,5			yes	
Cerebellum	yes	3	yes	yes	yes	
Molar tooth sign	yes		yes	yes	yes	
Hypoplasia of the cerebellum	yes	3	yes	yes	yes	
Reduced cerebellar foliation	yes	3		yes	yes	
Reduced sup. cerebellar peduncles	yes	3		yes	yes	
Reduced decussation of the cerebellar peduncles	yes	3		yes	yes	yes
Loss of granule layer		3			yes	
Disorganised Purkinje cells		3				yes
Eyes	yes	1		yes		
Coloboma	yes	6		yes		
Retinal degeneration	yes	6				
Loss of photoreceptor polarity		6				yes
Behaviours	yes	3	NA	yes		
Ataxia	yes	3		yes		
Hypotonia	yes			yes		
Irregular breathing	yes			yes		
Intellectual disability	yes			yes		
SKELETAL DEFECTS	yes	1,4,5	yes	yes	yes	
Polydactyly	yes	1,2,5	yes			
Syndactyly	yes	1,2				yes
Failure of ossification	yes	1,4				
Shortened long bones	yes	1,4	yes	?	yes	yes
Short ribs	yes	1	yes	rare		
VISCERA DEFECTS	yes	1			yes	
Abnormal situs	yes	2,5,6			yes	
Heart defects	yes			rare		
Kidney cysts	yes	1,5		rare		yes
Small lungs	yes	1		rare	yes	
Hepatic fibrosis	**yes**	1			yes	

Table 1- An abbreviated review of the phenotypes associated with a loss of *TALPID3/KIAA0586* [1-5, 7-15, 17, 18, 20, 22, 23, 26, 27, 31, 34-38]

Figure and Table Legends

Table 1- An abbreviated review of the phenotypes associated with a loss of *TALPID3/KIAA0586* [1-5, 7-15, 17, 18, 20, 22, 23, 26, 27, 31, 34-38]

1-7 indicate *TALPID3 mutant* alleles in model systems. 1=*talpid*^{3} chicken (null), 2= *Talpid3*$^{-/-}$ mouse (null), 3=Nestin-cre (null central nervous system), 4=Prx-cre (null in limb), 5=Maternal Zygotic talpid3$^{-/-}$ zebrafish (null), 6=Zygotic ta3$^{-/-}$ zebrafish (hypomorph/progressive loss), 7= siRNA Knockdown and CRISPR/Cas9 generated KIAA0586$^{-/-}$, human cells. CNS= central nervous system, NA= not appliciable, ?= unclear phenotype. Short Rib Polydactyly and Joubert Syndrome refer to the phenotypes observed in human patients with mutations in *TALPID3/KIAA0586*.

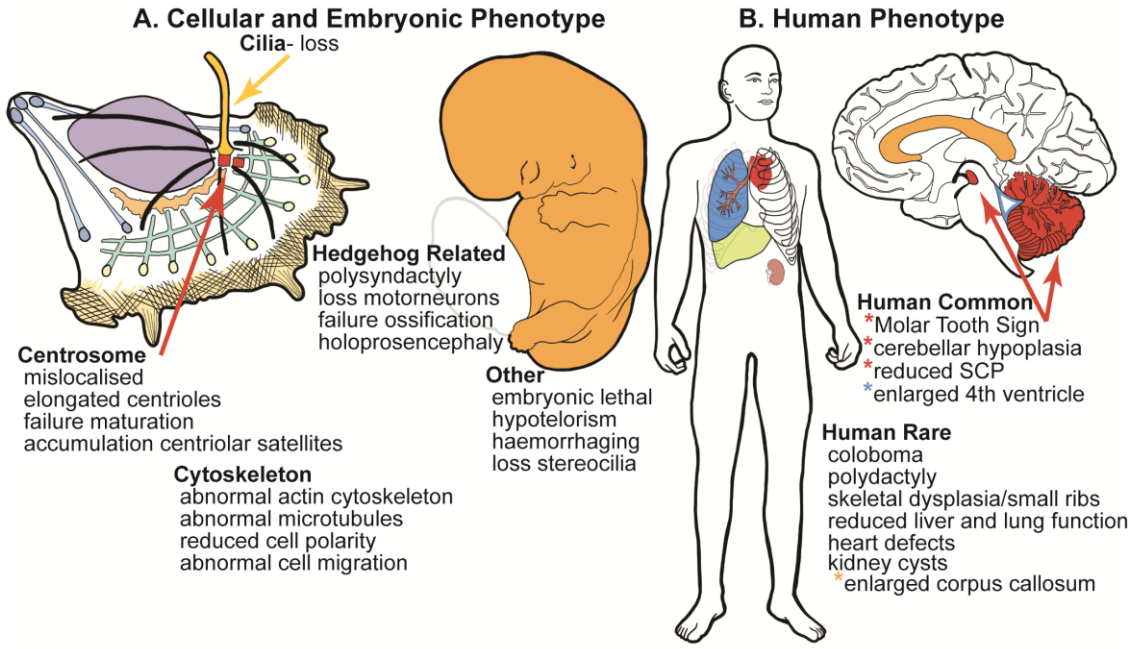

Figure 1- The phenotypes associated with a loss of TALPID3 in the cell, embryo and human. Figure 1A. The phenotypes associated with *TALPID3* are primarily caused by a loss of centrosome (red arrow) function and a loss of the cilia (yellow arrow)- an organelle dependent on normal centrosome function. Abnormal cellular phenotypes oberved in null *TALPID3* cells in culture and in the embryo are as listed. Homozygous null alleles cause an embryonic lethal phenotype in humans, mice, zebrafish and chicken embryos, characterised by the phenotypes listed and include those associated with disrupted Hedgehog signalling as well as phenotype which are independent of Hedgehog signalling. **Figure 1B**. In comparison with null embronic lethal phenotypes, viable humans with mutations in TALPID3 are likely to have hypomorphic mutations, which are usually compound heterozygous mutations. Common phenotypes in these patients include brain defects related to the cerebellum and associated structures (red arrows), presenting as a 'Molar Tooth Sign' which is characteristic of Joubert syndrome. More rarely a proportion of patients present with other 'ciliopathy' related phenotypes, such as polydactyly and other phenotypes listed.

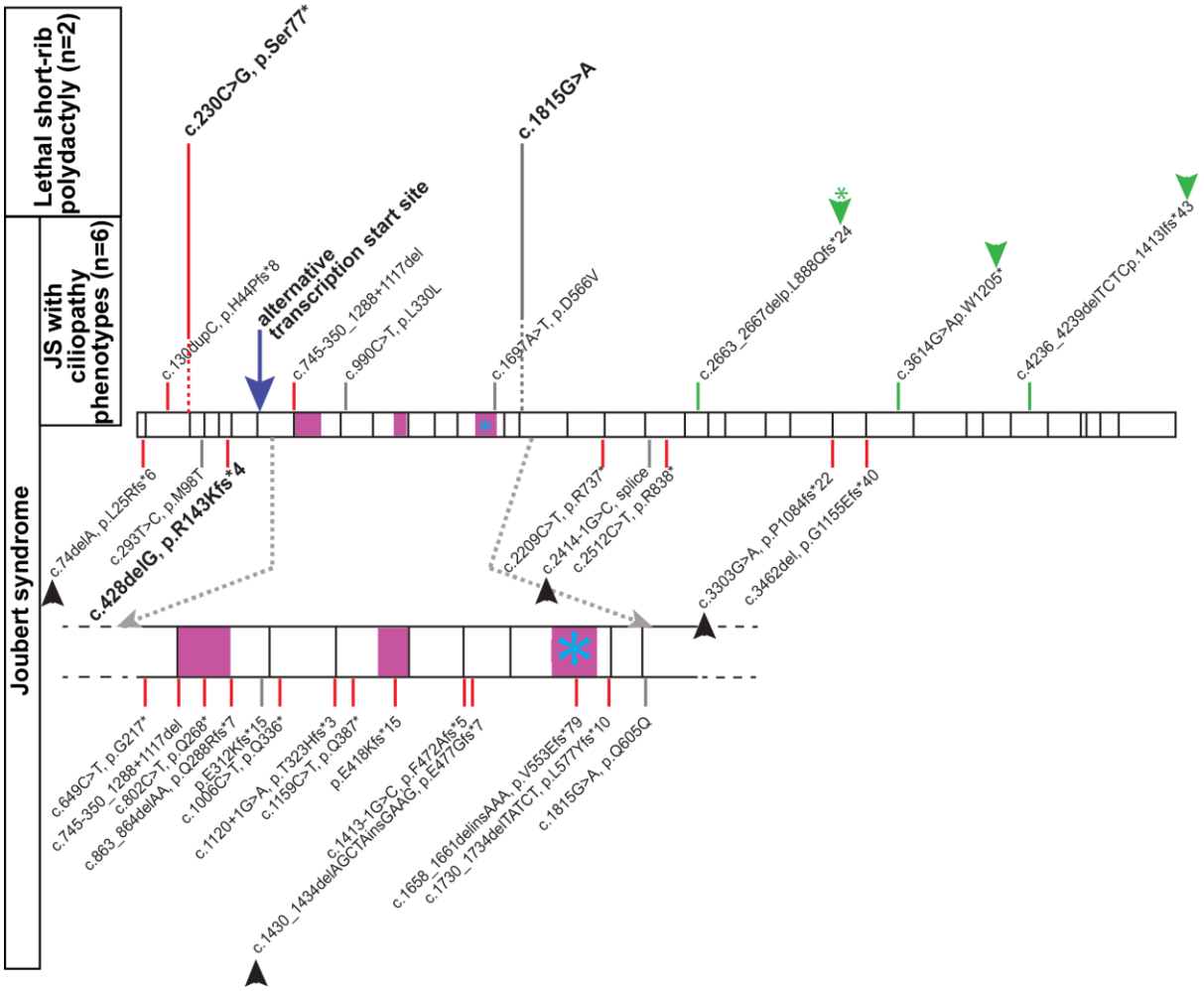

Figure 2.- The mutations in *TALPID3/KIAA0586* which cause human disease

Figure 2. Human *TALPID3/KIAA0586* mutations can be grouped into three main categories rare Lethal short-rib polydactyly (n=2; both are homozygous patients), rare Joubert syndrome with addition ciliopathy phenotypes (annotated above the gene structure) and relatively mild compound heterozygous mutations which cause Joubert syndrome (anotated below the gene structure). Green arrows heads represent rare single deleterious mutations, c.2663_2667delp.L888Qfs*24 has been shown to be essential for CP110 removal [26]. A hotspot for Joubert syndrome mutations, immediately prior to the essential coiled coil domain (blue asterisk) is shown in greater detail. The most common mutation, c.428delG,pR143Kfs*4, is annotated in bold and the potential alternative transcriptional start is shown by the blue arrow. Heterozygous mutations which do *not* occur with the common variant are marked by black

arrowheads. The mutations are annotated on a schematic of the human KIAA0586 protein. Black vertical lines represent exon boundaries, purple boxes mark the coiled coil domains, red vertical lines indicate mutations predicted to result in premature stop codons, grey vertical lines represent predicted splice variant mutations.

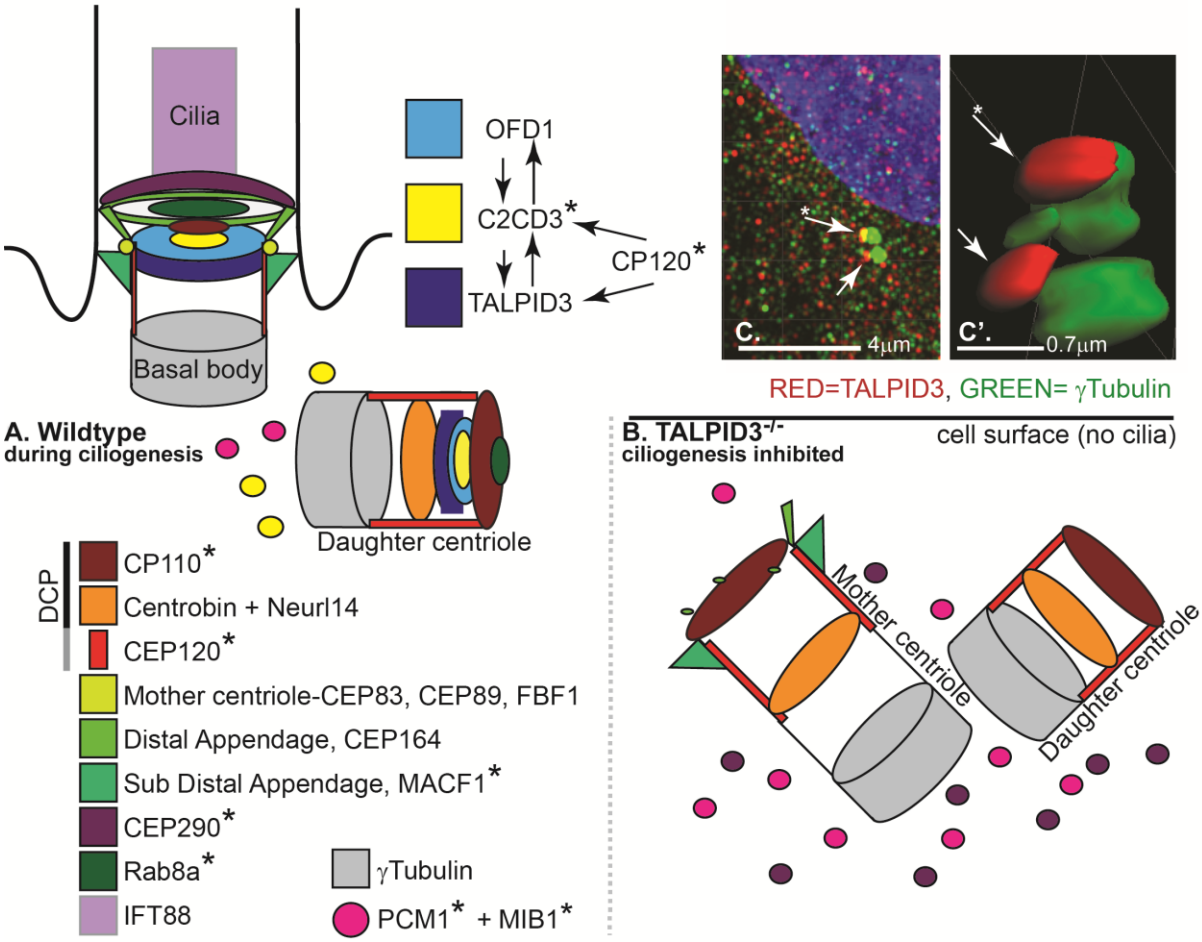

Figure 3- Model of the function of TALPID3 in the centrosome and the centrosome phenotypes caused by a loss of TALPID3

(A). TALPID3 interacts with CEP120 and C2CD3 to establish a TALPID3, C2CD3 and OFD1 complex at the distal ends of the mother and daughter centriole, prior to ciliogenesis. TALPID3 controls loss of DCP (daughter centriolar proteins; CEP120, CP110, Centrobin, Neurl14) and contributes in the acquisition of mother centriolar specific proteins (CEP83, CEP89, FBF1 module), distal appendage development including localisation of CEP164 (with C2CD3 and CEP120) and ciliary vescile binding (with Rab8a) during the maturation of the mother centriole towards ciliogenesis.TALPID3 binds to CP110, limiting localisation of CP110 on the mother centriole and limiting the length of daughter centriole length. PCM1 and C2CD3 are found in a low number of centriolar satellites around the centrioles **B.** A loss of TALPLID3 localisation from the centrioles results in a loss of C2CD3 and OFD1 and CEP120 is maintained at higher

daughter centriolar levels. Daughter centriolar proteins are maintained and localisation of mother centriolar proteins fails. While subdistal appendages develop, distal appendages are abnormal, CEP164 is disorganised, ciliary vesicles fail to dock. CP110 is also maintained at daughter centriolar levels and centrioles are elongated. Centrioles fail to migrate of orientate correctly to the cell surface. Centriolar satillites are abundant and inclide localisation of PCM1 and Cep290. * indicates known protein interaction with TALPID3.**C, C'.** Super resolution image and reconstruction of TALPID3 localisation (arrows, red) at the distal end of the centrosome via γTublin (green).

PHENOTYPE	Ciliopathy phenotype	Null TALPID3 Animal Model	Human Short-Rib Polydacyly	Human Joubert Syndrome	Hedgehog related	Cell polarity related defect
CELLULAR DEFECTS	yes	1, 2, 3, 5, 6, 7	yes			yes
Cilia absent or ciliated cells significantly reduced, size reduced		1, 2, 3, 5, 6, 7	yes	Yes [11]	causative of loss HH	yes
Abnormal centrosome/basal body location		1, 2, 3, 5, 6, 7				yes
Loss basal body subdistal appendage/markers (CEP164, CP120)		7				
Increase centriolar satellites		1,7				
Increased centriole length		1,7				
Failure of Distal Appendage		1 (reduced, CEP164 seen but abnormal), 7 (no CEP164)	CEP164 seen			
Abnormal microtubule cytoskeleton		1				yes
Abnormal actin cytoskeleton		1,7				yes
Mislocation of Golgi		1				yes
Mislocation of mitochondria		1,6				yes
Abnormal cell migration		1,2,3				yes
Abnormal Hedgehog signalling (loss *PTC1*, *GLI1* expression, mislocation of GLI protein)	yes	1,2,3,5	yes		yes	
Abnormal Wnt signalling	yes	3				
FACE AND HEAD DEFECTS	yes	5	yes	yes	yes	yes
Frontonasal prominence	yes	1,2	yes	yes	yes	
Hypotelorism	yes	1,2,5		yes	yes	
Midface shortening	yes			yes		
Long philtrum				yes		
Mandibular prominence	yes	1,2		yes	yes	
Micrognathia	yes	1		yes	yes	
Retrognathia	yes	1	yes			
Cleft tongue	yes		yes			
Abnormal dentition	yes		yes	yes	yes	
Maxillary prominence		1,2	yes		yes	
Cleft palate	yes		yes			
Abnormal dentition	yes			yes	yes	
Pituitary gland absence		1			yes	
CENTRAL NERVOUS SYSTEM DEFECTS	yes	1,2,3,5		yes	yes	
Hydrocephaly	yes	3	yes	yes		
Enlarged ventricles	yes	3	yes	yes		
Forebrain	yes	2,3	yes		yes	
Holoprosencephaly	yes	1,2,5			yes	
Midbrain	yes	1			yes	
Expansion of midbrain boundaries	yes	1,3			yes	
Hindbrain		1,3			yes	
Cerebellum	yes	3	yes	yes	yes	
Molar tooth sign	yes		yes	yes	yes	
Hypoplasia of the cerebellum	yes	3	yes	yes	yes	
Reduced cerebellar foliation	yes	3		yes	yes	
Reduced superior cerebellar peduncles	yes	3		yes	yes	
Reduced decussation of the cerebellar peduncles	yes	3		yes	yes	yes
Loss of granule layer		3			yes	
Disorganised Purkinje cells/abberrant connectivity		3				yes
Spinal cord	yes	2,5			yes	
Loss of ventral Hh dependent neurons	yes	1,2,5			yes	
Expansion dorsal neurons	yes	1,2			yes	
Eyes	yes	1		yes		
Coloboma	yes	6		yes		
Retinal degeneration	yes	6				

Loss of photoreceptor polarity		6				yes
Ectopic lens development		1				
Loss of stereocilia on hair cells	yes	1				yes
Behaviours	yes	3	NA	yes		
Ataxia	yes	3		yes		
Hypotonia	yes			yes		
Irregular breathing	yes			yes		
Intellectual disability	yes			yes		
AXIAL AND APPENDICULAR SKELETAL DEFECTS	yes	1,4,5	yes	yes	yes	
Limb	yes	1,2,5	yes	yes	yes	
Polydactyly	yes	1,2,5	yes			
Syndactyly	yes	1,2				yes
Shortened/curled body axis	partly	1,5			yes	yes
Skeleton	yes	1,4	yes		yes	
Failure of ossification	yes	1,4				
Mislocation of cell polarity marker (VANGL2)		1				yes
Misorientation of chondrocytes		1				yes
Shortened long bones	yes	1,4	yes	?	yes	yes
Short ribs	yes	1	yes	rare		
VISCERA DEFECTS	yes	1			yes	
Abnormal situs	yes	2,5,6			yes	
Heart defects	yes			rare		
Kidney cysts	yes	1,5		rare		yes
Small lungs	yes	1		rare	yes	
Hepatic fibrosis	yes	1			yes	
Gut patterning/innervation		1			yes	
MUSCLE DEFECTS		1,5			yes	
Abnormal myotome/muscle development		1,5			yes	
INTEGUMENTARY DEFECTS -Skin/Feather/Hair/Scales/Nails		1			yes	
Failure of appendage development		1			yes	
Lost of polarity of appendage		1				yes
VASCULAR DEFECTS		1,5			yes	
Ectopic blood vessels		1				
Haemorrhage		1,2,3,5				
Oedema		1,3				

Supplementary Table1. A comprehensive review of the phenotypes associated with a loss of *TALPID3/KIAA0586* [1-5, 7-15, 17, 18, 20, 22, 23, 26, 27, 31, 34-38]

1-7 indicate *TALPID3 mutant* alleles in model systems. 1=*talpid*^{3} chicken (null), 2= *Talpid3*$^{-/-}$ mouse (null), 3=Nestin-cre (null central nervous system), 4=Prx-cre (null in limb), 5=Maternal Zygotic talpid3$^{-/-}$ zebrafish (null), 6=Zygotic ta3$^{-/-}$ zebrafish (hypomorph/progressive loss), 7= siRNA Knockdown and CRISPR/Cas9 generated KIAA0586$^{-/-}$, human cells. CNS= central nervous system, NA= not appliciable, ?= unclear phenotype. Short Rib Polydactyly and Joubert Syndrome refer to the phenotypes observed in human patients with mutations in *TALPID3/KIAA0586*.